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Exogenous Indirect Photoinactivation of Bacterial Pathogens and Indicators in Water with Natural and
Synthetic Photosensitizers in Simulated Sunlight With Reduced UVB

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ABSTRACT

Aims: To investigate the UVB-independent and exogenous indirect photoinactivation of eight human health-relevant bacterial species in the presence of photosensitizers.

Methods and Results: Eight bacterial species were exposed to simulated sunlight with greatly reduced UVB light intensity in the presence of three synthetic photosensitizers and two natural photosensitizers. Inactivation curves were fit with shoulder-log linear or first order kinetic models, from which the presence of a shoulder and magnitude of inactivation rate constants were compared. 84% reduction in the UVB light intensity roughly matched a 72-95% reduction in the overall bacterial photoinactivation rate constants in sensitizer-free water. With the UVB light mostly reduced, the exogenous indirect mechanism contribution was evident for most bacteria and photosensitizers tested, although most prominently with the Gram-positive bacteria.

Conclusions: Results confirm the importance of UVB light in bacterial photoinactivation and, with the reduction of the UVB light intensity, that the Gram-positive bacteria are more vulnerable to the exogenous indirect mechanism than Gram-negative bacteria.

Significance and Impact of Study: UVB is the most important range of the sunlight spectrum for bacterial photoinactivation. In aquatic environments where photosensitizers are present and there is high UVB light attenuation, UVA and visible wavelengths can contribute to exogenous indirect photoinactivation.

Keywords: bacteria, sunlight, disinfection, photoinactivation, exogenous, sensitizers, reactive oxygen species, enterococci, *E. coli*, UVB, pathogens

INTRODUCTION

Sunlight driven growth, decay, and inhibition processes modulate concentrations of bacteria (Whitman *et al.* 2004; Enns *et al.* 2012), viruses (Wilhelm *et al.* 1998), algae (Heaney and Talling 1980), copepods (Leech *et al.* 2005), and many other living organisms in aquatic environments. While many processes utilize parts of the solar spectrum for beneficial use, there is a nearly universal, harmful effect of ultraviolet (UV) light exposure in the UVB range (280-320 nm) (Hader *et al.* 1998). In surface waters, much of the UVB exposure occurs within the top few centimeters before dissolved and particulate constituents, such as organic matter, attenuate UVB at greater depths (Arts *et al.* 2000; Davies-Colley *et al.* 2005). The less harmful UVA (320-400 nm) and visible light (400-700 nm) are attenuated to a smaller extent and therefore able to penetrate deeper into the water column than UVB (Arts *et al.* 2000; Davies-Colley *et al.* 2005). An example for the relative changes of light intensity at different wavelengths with depth for a natural water is shown in Figure 1.

Sunlight exposure of bacteria can result in photoinactivation. The rate of photoinactivation and the mechanism via which it occurs are believed to vary with the relative intensities of UV and visible light. Past studies have found UVB to be primarily responsible for bacterial photoinactivation, with UVA and visible light playing minor roles (Calkins and Barcelo 1982). Photoinactivation of bacteria may occur via three different mechanisms: endogenous direct, endogenous indirect, and exogenous indirect (Davies-Colley *et al.* 1999; Davies-Colley *et al.* 2000). Endogenous direct photoinactivation results from damage to vital cellular components, such as DNA, principally by UVB light (Malloy *et al.* 1997; Schuch and Menck 2010), but also possibly due to UVA light (Schuch and Menck 2010). In the absence of UVA and UVB, inactivation has been found to be mainly driven by indirect processes (Davies-Colley *et al.* 2000).

In indirect photoinactivation, a photosensitizer inside (endogenous) or outside (exogenous) the bacterial cell absorbs light and transfers energy or electrons to form reactive species that can damage cellular components. Various molecules occurring within living organisms, for example flavins, porphyrins, bilirubin or chlorophyll, can act as endogenous sensitizers while dissolved natural organic matter (DOM), i.e. mainly humic and fulvic acids, can act as exogenous sensitizers (Curtis *et al.* 1992; Sinton *et al.* 2002).

To date, there is a general understanding that the relative importance of each of the three photoinactivation mechanisms depends on the relative magnitudes of the UVB, UVA, and visible light in a water column (Davies-Colley *et al.* 2005), the reactivity and properties of the exogenous photosensitizers (Rosado-Lausell *et al.* 2013), and the susceptibility of the bacteria to each of the three mechanisms (Nguyen *et al.* 2015). In a recent study using full spectrum sunlight, the exogenous indirect photoinactivation mechanism contributed significantly to photoinactivation of Gram-positive bacteria but generally not to photoinactivation of Gram-negative bacteria (Maraccini *et al.* 2016b). Additionally, the exogenous indirect photoinactivation was only observed when using synthetic photosensitizer that generated reactive oxygen species (ROS) at or above (up to 3 fold more) the upper concentration limit typically found in sunlit aquatic environments. When using environmentally sourced photosensitizers that generated ROS concentrations at levels naturally occurring in the sunlit aquatic environment, photoinactivation was instead unaffected or slightly suppressed (Maraccini *et al.* 2016b). In some aquatic environments, UVB may be suppressed relative to the rest of the natural light spectrum, such as in wetlands, lakes, or coastal waters with high dissolved organic carbon (DOC) concentrations (Arts *et al.* 2000). Under those UVB suppressed scenarios, studies have investigated the exogenous indirect photoinactivation mechanism in wetland waters with high DOC concentrations for fecal indicator bacteria *Escherichia coli* and enterococci (Kadir and Nelson 2014; Nguyen *et al.* 2015). However, the inactivating effects of UVA and visible light for actual bacterial pathogens under such conditions have not been assessed so far.

The purpose of this study was to determine the relative importance of the exogenous indirect photoinactivation mechanism in aquatic environments with high UVB light attenuation, where photoinactivation is driven by the UVA and visible light portions of the solar spectrum. We conducted photoinactivation experiments with eight health-relevant bacterial species (*Bacteroides thetaiotaomicron*, *Campylobacter jejuni*, *Enterococcus faecalis*, *E. coli* K12, *E. coli* O157:H7, *Salmonella enterica* serovar Typhimurium LT2, *Staphylococcus aureus*, and *Streptococcus bovis*) in a solar simulator equipped with a UVB cutoff filter. The bacterial species were chosen as each one is a pathogen or correlates to waterborne illness risk. Synthetic and natural sensitizers were added individually to buffered aqueous solutions spiked with known bacterial concentrations. The goal of the study was to broadly identify if exogenous indirect photoinactivation is important in UVB-light reduced environments. The findings of this study should aid in determining how to best model bacterial decay in surface waters in the presence of sunlight.

MATERIALS AND METHODS

Bacterial cultivation. Bacteria were cultivated as described in a previous publication (Maraccini *et al.* 2016b). Briefly, facultative anaerobes *Enterococcus faecalis* (ATCC 19433), *Escherichia coli* K12 (ATCC 10798), *Escherichia coli* O157:H7 (ATCC 43895), *Staphylococcus aureus* (ATCC 25923), *Streptococcus bovis* (ATCC 33317), and the attenuated strain *Salmonella enterica* serovar Typhimurium LT2 obtained from the Falkow Lab of Stanford University were grown in aerated chemostats with tryptic soy broth. The chemostats were first immersed in a 37 °C water bath, held at a constant dilution rate (0.005 min⁻¹), then inoculated with a single bacterial species. After the optical density stabilized (~8 hours), cells were harvested from the chemostats to serve as the seed stock for the photoinactivation experiments. Seed stocks of facultative anaerobes were generated from chemostats to minimize the variability among replicates (Maraccini *et al.* 2015).

Bacteroides thetaiotaomicron (ATCC 29741) and *Campylobacter jejuni* (ATCC 29428) were grown in batch cultures in anaerobic and microaerophilic environments, respectively, with modified TYG medium and brucella broth, respectively. After 24 and 48 hours of incubation for *B. thetaiotaomicron* and *C. jejuni*, respectively, cells were harvested to serve as the seed stock. Chemostats could not be used for microaerophiles and obligate anaerobes due to the difficulty of controlling the atmosphere in the constructed setup. Bacteria were harvested by pelleting and re-suspending the cells in 1 mmol l⁻¹ carbonate buffer saline (CBS, pH 7.64).

Each bacterial strain used in this study was a common pathogen or fecal indicator organism and represented either Gram negative or positive bacteria (Maraccini *et al.* 2016b).

Experimental solutions and procedures. Experimental solutions and procedures are described in a previous publication (Maraccini *et al.* 2016b), with a few alterations. Briefly, photosensitizer stock solutions were added to separate beakers to reach final concentrations of 0.5 μmol l⁻¹ methylene blue (MB), 200 mg l⁻¹ as nitrite, 0.5 μmol l⁻¹ rose bengal (RB), 20 mg C l⁻¹ Suwanee River Natural Organic Matter (SRNOM), and 20 mg C l⁻¹ dissolved organic matter isolated from a treatment wetland (iDOM). All solutions were prepared with a 1 mmol l⁻¹ CBS solution. These photosensitizers were chosen to represent both natural (SRNOM and iDOM) and synthetic sensitizers (MB, RB, and nitrite), different surface charges (anionic for SRNOM, iDOM, RB, and nitrite; cationic for MB), and varying concentrations of reactive oxygen species generated (Maraccini *et al.* 2016b). Nitrite, while found in natural environments, is unlikely to be measured at the high concentrations used in the experiment (Eddy and Williams 1987). As the nitrite used was a chemical additive, and the natural sensitizers were harvested from the natural environment for use in this study, nitrite is hereby viewed as a synthetic photosensitizer. A single species of bacteria was then added to a beaker containing one of the aforementioned photosensitizers. Experiments with iDOM and methylene blue were limited to representative Gram-negative and Gram-positive organisms: *E. coli* K12 and *Ent. faecalis*. The bacterial concentrations at the start of each experiment were 3.3 x 10⁴ – 5.6 x 10⁶ colony forming units (CFU) ml⁻¹.

for *B. thetaiotaomicron*, $9.2 \times 10^5 - 5.8 \times 10^6$ CFU ml⁻¹ for *C. jejuni*, $5.4 \times 10^6 - 2.8 \times 10^7$ CFU ml⁻¹ for *E. coli* K12, $4.3 \times 10^6 - 1.3 \times 10^7$ CFU ml⁻¹ for *E. coli* O157:H7, $1.1 \times 10^6 - 1.1 \times 10^7$ CFU ml⁻¹ for *Ent. faecalis*, $1.4 \times 10^7 - 2.8 \times 10^7$ CFU ml⁻¹ for *S. enterica*, $2.0 \times 10^7 - 4.1 \times 10^7$ CFU ml⁻¹ for *Staph. aureus*, and $2.4 \times 10^4 - 1.8 \times 10^6$ CFU ml⁻¹ for *Strep. bovis* (Table S1).

Beakers were placed in a recirculating water bath set at 15 °C in a solar simulator (Atlas Suntest CPS+; Linsengericht-Altenhaßlau, Germany) that was equipped with a coated quartz filter and a UV special glass filter and set at 400 W m⁻². An additional UVB cutoff filter (FSQ-WG320, Newport, Franklin, MA) was placed atop the beakers. The UVB cutoff filter reduced irradiance at 320 nm by ~50%, at 310 nm by ~90%, and any wavelength less than 300 nm by >99.9% as compared to irradiance without the filter (Figure 2). The net reduction in incident UVB light intensity from 280-320 nm was 84%.

Beakers were stirred continuously. Dark control experiments consisted of an additional beaker containing the same bacterial species but covered with foil and placed in the same recirculating water bath to run in parallel with the beakers exposed to the simulated sunlight. Dark controls were run both in the presence and absence of photosensitizers.

Bacteria were enumerated over the course of the light irradiation by aseptically drawing 0.5 ml samples from the beakers. Samples were taken anywhere from 2 minutes to 3.5 hours depending on how quickly the bacteria lost culturability. Appropriate dilutions were then spread plated in duplicate on tryptic soy agar for facultative anaerobes, brucella agar for *C. jejuni*, and brain heart infusion agar supplemented with 5% horse blood for *B. thetaiotaomicron*.

Sensitizer-containing solutions were run in parallel with sensitizer-free solutions inoculated with bacteria from the same chemostat or batch culture for later pairwise comparisons. All experiments were performed in triplicate.

Chemical Probes. The steady-state bulk concentrations of singlet oxygen (¹O₂) and hydroxyl radical (·OH) were measured indirectly with probe compounds furfuryl alcohol and phenol, respectively,

as described previously (Maraccini *et al.* 2016b). The probe compound tests were performed with and without the UVB cutoff filter atop the beakers.

Data Analysis. Individual decay curves were fitted with a shoulder-log linear model (van Boekel 2002):

$$\frac{C(t)}{C_0} = e^{-kt} \left(\frac{e^{kS}}{1 + (e^{kS} - 1)e^{-kt}} \right) \quad (1)$$

where t is time (min), C (CFU ml⁻¹) is the measured concentration at time t , C_0 is the measured concentration at time $t = 0$, S (min) is the shoulder or lag time over which there is minimal inactivation of the bacteria, and k (min⁻¹) is the rate constant for the log linear portion of the inactivation curve after completion of the lag time. All fitted values are non-negative. If the 95% confidence interval of the shoulder length, S , crossed zero, then the inactivation data were refit using a simpler first order decay model (Equation 1 with $S = 0$):

$$\frac{C(t)}{C_0} = e^{-kt} \quad (2)$$

Fit parameters, and their standard deviations and 95% confidence intervals were obtained using IGOR PRO (WaveMetrics Inc., Lake Oswego, OR). The quality of each fitting was determined by a Pearson's χ^2 test for goodness of fit.

The rate constant k , in units of time, was corrected for UVB light screening and converted to \hat{k} , in units of m² MJ⁻¹, by dividing by the depth-averaged UVB light intensity, $\langle I_{UVB} \rangle_z$ [Wm⁻²], and completing the necessary unit conversion:

$$\hat{k} = \frac{k}{\langle I_{UVB} \rangle_z} \quad (3)$$

The shoulder length S , in units of time, was corrected for UVB light screening and converted to \hat{S} , in

units of MJ m⁻², by multiplying by <I_{UVB}>_z and completing the necessary unit conversion:

$$\hat{S} = S * \langle I_{UVB} \rangle_z \quad (4)$$

As detailed in previous publications (Schwarzenbach *et al.* 2002; Grandbois *et al.* 2008), <I_{UVB}>_z was found through the following equations:

$$\langle I_{UVB} \rangle_z = \sum_{280-320 \text{ nm}} \langle I_{\lambda} \rangle_z \quad (5)$$

$$\langle I_{\lambda} \rangle_z = I_{\lambda,0} \frac{(1 - 10^{-\alpha_{\lambda} z})}{2.303 \alpha_{\lambda} z} \quad (6)$$

where <I_λ>_z is the depth-averaged light intensity within the solution at a given wavelength, I_{λ,0} is the light irradiance at a given wavelength at the surface, α_λ is the light attenuation at a given wavelength, and z is the total depth of the solution. The I_{λ,0} was measured using a spectroradiometer (ILT950; International Light, Peabody, MA). The absorbance experimental solutions were measured using spectrophotometer (Uvikon XL, Research Instruments International, San Diego, CA) against a reference solution of deionized water, with the resulting absorbance at each wavelength representing α_λ. The absorbance was measured for all experimental solutions and used to calculate <I_{UVB}>_z.

The observed \hat{k} was assumed to be the sum of the endogenous photoinactivation rate constant, $\hat{k}_{endogenous}$, and the exogenous photoinactivation rate constant, $\hat{k}_{exogenous}$.

$$\hat{k} = \hat{k}_{endogenous} + \hat{k}_{exogenous} \quad (7)$$

$\hat{k}_{endogenous}$ was defined as the average \hat{k} values between the sensitizer-free control replicates for each bacterial species. \hat{k} values were compared pairwise between treatments (i.e., experiments where the aqueous solution contained a photosensitizer) and controls (i.e., experiments not containing a photosensitizer) for organisms from the same chemostat or batch replicate using a t-test. If \hat{k} from the

treatment was significantly greater ($P < 0.05$) than \hat{k} for the control for at least 2 of the 3 replicates, then the sensitizer was deemed to promote exogenous photoinactivation. In those cases, $\hat{k}_{exogenous}$ was calculated as the difference between the average \hat{k} for the treatments and the $\hat{k}_{endogenous}$. If the sensitizer was deemed not to cause exogenous photoinactivation, $\hat{k}_{exogenous}$ was set equal to zero.

$\hat{k}_{exogenous}$ values were compared between bacteria groups (Gram-negative facultative anaerobe, Gram-positive facultative anaerobe, Gram-Negative facultative microaerophile, Gram-negative obligate anaerobe) and experimental solutions and the interaction between bacteria and the experimental solutions using analysis of variance (ANOVA) and Fisher least square difference (LSD) post hoc tests. Linear regression assessed whether $\hat{k}_{exogenous}$ correlated with steady state concentrations of singlet oxygen or hydroxyl radical. $\hat{k}_{exogenous}$ values were \log_{10} transformed for the analysis to achieve normality. A value of 0 was used for $\log_{10}(\hat{k}_{exogenous})$ when $\hat{k}_{exogenous}$ equaled zero. The presence or absence of a shoulder was compared between bacterial species and experimental solutions using contingency tables and χ^2 tests. All statistics were completed using Statplus:mac (AnalystSoft Inc., version 2009).

RESULTS

Curve fits. Of the 116 individual experiments fit with either a shoulder-log linear model or first-order decay model (Figure S1, Table S1), the Pearson's χ^2 test values were greater than 0.05 for 114 (98%) (Table S2), indicating the models were a good fit for the data. The k from log-linear fittings of the dark controls was not significantly different from zero as analyzed by linear regression ($P > 0.05$, data not shown), meaning there was no significant dark inactivation over the course of the experiments.

A shoulder was observed for 89 of the 116 inactivation curves (Table S2). The remaining 27 data series did not require a shoulder and were fit with the simplified model. All experiments conducted with *Strep. bovis* (n=12) had shoulders. 95% of the experiments conducted with *Ent. faecalis* (n=21) had a shoulder, as did 93% for *Staph. aureus* (n=14), 83% for *E. coli* O157:H7 (n=12), 77% for *E. coli* K12 (n=22), 75% for *S. enterica* (n=12), 58% for *C. jejuni* (n=12), and only 8% for *B. thetaiotaomicron* (n=12). The presence of a shoulder significantly varied between organisms (ANOVA, $P < 0.05$), but not between photosensitizer present in solution or the interaction (organism x photosensitizer present) of the independent variables (ANOVA, $P > 0.05$).

Photoinactivation in the absence of photosensitizers. In sensitizer-free CBS controls, \hat{k} values ranged from $573 \text{ m}^2 \text{ MJ}^{-1}$ to $7655 \text{ m}^2 \text{ MJ}^{-1}$ with a median of $1234 \text{ m}^2 \text{ MJ}^{-1}$ (Table S2). $\hat{k}_{\text{endogenous}}$, which was the average of at least three replicate experiments in a sensitizer-free solution for each bacterium, was compared between experiments with and without the UVB cutoff filter (Table 1, includes previously published values (Maraccini *et al.* 2016b)). The percent reduction in $k_{\text{endogenous}}$ (units of time) with the application of the UVB cutoff filter was on average 89%, with the largest reduction being 95% with *Strep. bovis* and the smallest 72% with *Staph. aureus*.

Effect of photosensitizers on photoinactivation and comparison of $\hat{k}_{\text{exogenous}}$. The decay of the probe compounds in each sensitizer solution was compared by linear regression under light irradiation with and without application of the UVB cutoff filter. The difference in slopes was not significant ($P > 0.05$), therefore the steady state concentrations of singlet oxygen and hydroxyl radical were the same with the UVB cutoff filter as without the filter, which was previously reported in Maraccini *et al.* (2016b).

The presence of a photosensitizer triggered exogenous photoinactivation in 22 of the 28 bacteria-photosensitizer treatments tested (Figure 3). For *Ent. faecalis*, the presence of MB, RB, nitrite, SRNOM, and iDOM increased \hat{k} compared to the CBS control. For *Staph. aureus* and *Strep. bovis*, the presence of

MB, RB, and nitrite increased \hat{k} . For *S. enterica*, nitrite and SRNOM increased \hat{k} . For *E. coli* K12, MB, nitrite, and SRNOM increased \hat{k} . For *E. coli* O157:H7, nitrite increased \hat{k} . For *B. thetaiotaomicron*, RB and nitrite increased \hat{k} . For *C. jejuni*, RB and SRNOM increased \hat{k} .

$\hat{k}_{exogenous}$ ranged from 324 m² MJ⁻¹ to 89599 m² MJ⁻¹ with a median of 3994 m² MJ⁻¹ (Table S3).

ANOVA was used to explore trends in $\hat{k}_{exogenous}$ across different experimental treatments. On average, $\hat{k}_{exogenous}$ significantly varied between organisms (ANOVA, $P < 0.05$), but not between photosensitizer present in solution or the interaction (organism x photosensitizer present) of the independent variables (ANOVA, $P > 0.05$). Linear regression also indicated that $\hat{k}_{exogenous}$ did not correlate with concentrations of singlet oxygen or hydroxyl radicals generated by the photosensitizers ($P > 0.05$). Post hoc analysis indicated $\hat{k}_{exogenous}$ was larger for Gram-positive facultative anaerobes (*Ent. faecalis*, *Staph. aureus*, *Strep. bovis*) than for Gram-negative facultative anaerobes (*E. coli* K12, *E. coli* O157:H7, *S. enterica*) and the Gram-negative obligate anaerobe *B. thetaiotaomicron* ($P < 0.05$). Post hoc analysis also indicated the $\hat{k}_{exogenous}$ with RB was larger for the Gram-positive facultative anaerobes than for Gram-negative facultative anaerobes. This can be clearly seen for the results with *Ent. faecalis* (Figure 3).

DISCUSSION

UVB is primarily responsible for the photoinactivation of bacteria under unmodified simulated sunlight. Comparing $k_{endogenous}$ from experiments using UVB-reduced simulated sunlight to $k_{endogenous}$ from experiments using unmodified simulated sunlight, as reported in a companion study using the same health-relevant bacteria (Maraccini *et al.* 2016b), the percent reduction in $k_{endogenous}$ roughly equated to the percent reduction in the net UVB light intensity (84%). The large reduction in $k_{endogenous}$ suggests that UVB was primarily responsible for photoinactivation under unmodified solar simulated light, in

agreement with past studies (Sinton *et al.* 1994). In addition, when $k_{\text{endogenous}}$ was corrected for UVB light screening, the magnitudes of $\hat{k}_{\text{endogenous}}$ with (this study) and without (Maraccini *et al.* 2016b) the UVB cutoff filter were of the same order of magnitude. Our results provide further evidence that the UVB irradiation is the primary means of photoinactivation under normal sunlight conditions.

The addition of photosensitizers to clear water increased \hat{k} in 22 of 28 bacteria-photosensitizer treatments relative to its value in clear water. \hat{k} of all Gram-positive bacteria increased with every photosensitizer tested, while at least one photosensitizer increased \hat{k} for each Gram-negative bacterium tested. The ROS concentrations generated with the UVB-reduced light were not significantly different from the ROS concentration generated under unmodified solar simulator irradiation (data not shown). The application of the UVB cutoff filter still allowed for passage of the longer light wavelengths (>320 nm) and, because the synthetic photosensitizers had absorbance peaks outside the UVB spectrum (Figure S2), the light at wavelengths responsible for the ROS generation passed through the UVB cutoff filter and was absorbed by the photosensitizers. Because the natural sensitizers absorbed strongly in the UVB region, the addition of the UVB cutoff filter could have reduced some ROS generation. However, past work has shown the potential for DOM to generate high ROS concentrations using UVB blocked light (Rosado-Lausell *et al.* 2013).

Irradiation with UVB-reduced light resulted in a higher average $\hat{k}_{\text{exogenous}}$ for Gram-positive bacteria than Gram-negative bacteria *B. thetaiotaomicron*, *E. coli* K12, *E. coli* O157:H7, and *S. enterica*. Similar to the trends observed using unmodified solar simulator irradiation (Maraccini *et al.* 2016b) and during photodynamic therapy (Malik *et al.* 1992; Demidova *et al.* 2005), the enhancing effect of the sensitizers to photoinactivation occurred with greater regularity and at higher magnitudes with Gram-positive bacteria than Gram-negative bacteria. In Gram-negative bacteria, the additional outer membrane may confer some protection against exogenous photoinactivation (Dahl *et al.* 1988; Mamone *et al.* 2014)

On average, there was no statistical difference in the magnitude of $\hat{k}_{exogenous}$ measured in experiments conducted with different photosensitizers. The addition of methylene blue resulted in the highest $\hat{k}_{exogenous}$ among any photosensitizer tested; however, methylene blue was only tested on *E. coli* K12 and *Ent. faecalis*. The addition of rose bengal resulted in the highest or second highest $\hat{k}_{exogenous}$ for all bacteria tested except the Gram-Negative facultative anaerobes (*E. coli* K12, *E. coli* O157:H7, *S. enterica*), where the $\hat{k}_{exogenous}$ measured was either zero or very small. Methylene blue, a cationic photosensitizer, may more easily adsorb to and penetrate cell walls and membranes than anionic photosensitizers, such as rose bengal, thereby generating ROS closer to vital cellular components (Taheri-Araghi and Ha 2007; Jiang *et al.* 2008; Alves *et al.* 2009). Rose bengal generated approximately 10 times the singlet oxygen as methylene blue at the concentrations tested (Maraccini *et al.* 2016b). However, rose bengal had a neutral or minimal effect on Gram-negative facultative anaerobes, likely as a result from the added protection provided by the outer membrane that Gram-positive bacteria do not have (Dahl *et al.* 1988; Mamone *et al.* 2014). The natural photosensitizers enhanced photoinactivation for 8 of the 10 bacteria-natural photosensitizer pairs tested, although the resulting $\hat{k}_{exogenous}$ was typically among the lowest $\hat{k}_{exogenous}$ observed. The anionic surface charge at circumneutral pH (Au *et al.* 2011) and large size, relative to the synthetic photosensitizers, may have prevented the natural sensitizers from generating ROS close enough to the bacterial targets to induce more significant indirect photoinactivation (Latch and McNeill 2006; Kohn *et al.* 2007). It is possible that other DOMs with varying sizes, charges, and ROS-generating potentials could induce a greater magnitude of indirect photoinactivation.

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microaerophilic *C. jejuni*. While results indicate that cellular respiration or the environment in which the bacteria were cultured (aerobic, microaerobic, anaerobic) may have contributed to the presence or absence of a shoulder, past work has been unable to predict the occurrence of a shoulder (Cebrián *et al.* 2010). A shoulder may arise due to the need to inactivate multiple targets within a bacterial cell or multiple bacterial cells in a cell aggregate before inactivation is observed (Sinton *et al.* 1994; Sinton *et al.* 1999). A shoulder may also arise due to a diversity of resistance to photoinactivation among the bacterial culture (Najm 2006) or from a threshold effect, for which a cell can withstand a certain level of stress before death (Thurston-Enriquez *et al.* 2003; Cebrián *et al.* 2010). The results are consistent with past work with UVB-reduced light in which *Ent. faecalis* displayed a shoulder (Maraccini *et al.* 2015). Future work will need to address how to translate shoulders observed in laboratory experiments to modeling efforts in sunlit, surface waters and to determine whether the shoulder is a laboratory artifact or intrinsic to bacterial photoinactivation kinetics.

The photoinactivation rate constants reported herein were corrected for UVB light screening (280-320 nm), although other studies have corrected rate constants using UVA+UVB light (280-400 nm) (for example, Romero *et al.* 2011). Our logic for our choice to correct for UVB alone is as follows. We know UVB is important for direct and indirect endogenous bacterial photoinactivation. We wanted to compare rate constants when only direct photoinactivation was possible (clear water) to those when the additional indirect exogenous mechanism was possible (water with sensitizers). By correcting for UVB, we account for light screening that would impact the endogenous mechanisms, which would allow identification of additional mechanisms present, such as the exogenous indirect mechanism. Including the fluence from UVA light in the calculations of \hat{k} (see Tables S4 and S5 and Figure S3) had minimal changes on the conclusions described here. Therefore, the general trends found when correcting for UVB light screening described in the results still hold. When considering both UVB + UVA in the light screening calculations the following changes to the results reported using UVB alone were found. Three photosensitizer-bacteria pairings (*E. coli* K12 with SRNOM and iDOM and *Ent. faecalis* with SRNOM)

that exhibited higher \hat{k} than in the clear control no longer showed this result after correcting for both UVA and UVB. Thus, correcting for UVA and UVB resulted in increasing \hat{k} for 19 of 28 bacteria-photosensitizer combinations, compared to 22 out of 28 when correcting for UVB only. Additionally, including the fluence from UVA light in the calculations resulted in significantly decreased \hat{k} for the photosensitizer-bacteria pairing SRNOM with *E. coli* O157:H7 relative to the control.

There are some limitations to this study. The results regarding the importance of the exogenous indirect photoinactivation mechanism may be limited to surface waters with higher dissolved organic carbon (DOC) where UVB is quickly absorbed by DOC and visible light are able to penetrate to deeper depths (Arts *et al.* 2000). Also, the experiments were performed with lab cultured bacteria in buffered solutions, which may result in different photoinactivation kinetics than for indigenous bacteria (Fisher *et al.* 2012) in natural waters (Rosado-Lausell *et al.* 2013). Yet, the aim of this study focused on systematically assessing the exogenous indirect photoinactivation of a number of bacterial species in the presence of a select set of photosensitizers. Thus, this work was only possible in the highly controlled settings of this study. Lastly, there were other reactive species potentially responsible for exogenous indirect photoinactivation that we did not consider, such as triplet state DOM. A previous study correlated concentrations of triplet state NOM with the exogenous indirect photoinactivation rate constants of rotavirus (Rosado-Lausell *et al.* 2013; Romero-Maraccini *et al.* 2013); however, to date, the same correlation has not been made between triplet state NOM and bacteria. An interesting area for future research is to mechanistically investigate at a molecular level exogenous indirect bacterial photoinactivation.

The exogenous indirect mechanism may be a major contributor to bacterial photoinactivation whenever UVB light intensity is reduced and more so for Gram-positive bacteria. Based on the findings of this study, predictive models of bacterial attenuation in sunlit surface waters should put substantial weight into the UVB light intensity experienced by the bacteria and incorporate the exogenous

mechanism whenever the UVB is greatly diminished, particularly for Gram-positive bacteria. It should be noted that the exogenous photoinactivation rate constants found herein may not be directly applied to other aquatic environments. For each bacterium, the magnitude of the exogenous photoinactivation rate constant varied from photosensitizer to photosensitizer, indicating a differential dependence on the bacterial affinity to and the ROS concentration generated by the particular photosensitizer.

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CONFLICTS OF INTEREST

No conflict of interest declared.

REFERENCES

Alves, E., Costa, L., Carvalho, C.M.B., Tomé, J.P.C., Faustino, M.A., Neves, M.G.P.M.S., Tomé, A.C., Cavaleiro, J.A.S., Cunha, A., and Almeida, A., 2009. Charge effect on the photoinactivation of Gram-negative and Gram-positive bacteria by cationic meso-substituted porphyrins. *BMC Microbiol*, 9, p.70.

- Arts, M.T., Robarts, R.D., Kasai, F., Waiser, M.J., Tumber, V.P., Plante, A.J., Rai, H., and de Lange, H.J., 2000. The attenuation of ultraviolet radiation in high dissolved organic carbon waters of wetlands and lakes on the northern Great Plains. *Limnol Oceanogr*, 45(2), pp.292–299.
- Au, K.K., Alpert, S.M. and Pernitsky, D.J., 2011. Particle and natural organic matter removal in drinking water. In *Operational Control of Coagulation and Filtration Processes - Manual of Water Supply Practices, M37 (3rd Edition)*. pp. 1–16.
- Calkins, J. and Barcelo, J.A., 1982. Action Spectra. In J. Calkins, ed. *The role of solar ultraviolet radiation in marine ecosystems*. New York: Plenum Press, pp. 143–150.
- Cebrián, G., Michiels, C.W., Mañas, P., and Condón, S., 2010. Biological approach to modeling of *Staphylococcus aureus* high-hydrostatic-pressure inactivation kinetics. *Appl Environ Microbiol*, 76(21), pp.6982–90.
- Curtis, T.P., Mara, D.D. and Silva, S.A., 1992. Influence of pH, oxygen, and humic substances on ability of sunlight to damage fecal coliforms in waste stabilization pond water. *Appl Environ Microbiol*, 58(4), pp.1335–43.
- Dahl, T., Midden, W.R. and Neckers, D.C., 1988. Comparison of photodynamic action by rose bengal in Gram-positive and Gram-negative bacteria. *Photochem Photobio*, 48(5), pp.607–612.
- Davies-Colley, R.J., Donnison, A.M., Speed, D.J., Ross, C.M., and Nagels, J.W., 1999. Inactivation of faecal indicator micro-organisms in waste stabilisation ponds: interactions of environmental factors with sunlight. *Water Res*, 33(5), pp.1220–1230.
- Davies-Colley, R.J., Craggs, R.J., Park, J., and Nagels, J.W., 2005. Optical characteristics of waste stabilisation ponds: Recommendations for monitoring. *Water Sci Technol*, 51, pp.153–161.
- Davies-Colley, R.J., Donnison, A.M. and Speed, D.J., 2000. Towards a mechanistic understanding of pond disinfection. *Water Sci Technol*, 42(10-11), pp.149–158.
- Demidova, T.N., Gad, F., Zahra, T., Francis, K.P., and Hamblin, M.R., 2005. Monitoring photodynamic therapy of localized infections by bioluminescence imaging of genetically engineered bacteria. *J Photochem Photobio B*, 81(1), pp.15–25.
- Eddy, F.B. and Williams, E.M., 1987. Nitrite and freshwater fish. *Chem Ecol*, 3, pp.1–38.
- Enns, A.A., Vogel, L.J., Abdelzaher, A.M., Solo-Gabriele, H.M., Plano, L.R.W., Gidley, M.L., Phillips, M.C., Klaus, J.S., *et al.*, 2012. Spatial and temporal variation in indicator microbe sampling is influential in beach management decisions. *Water Res*, 46(7), pp.2237–2246.
- Fisher, M.B., Iriarte, M. and Nelson, K.L., 2012. Solar water disinfection (SODIS) of *Escherichia coli*, *Enterococcus* spp., and MS2 coliphage: effects of additives and alternative container materials. *Water Res*, 46(6), pp.1745–54.
- Grandbois, M., Latch, D.E. and McNeill, K., 2008. Microheterogeneous concentrations of singlet oxygen in natural organic matter isolate solutions. *Environ Sci Rechnol*, 42(24), pp.9184–90.
- Hader, D.P., Kumar, H.D., Smith, R.C., and Worrest, R.C., 1998. Effects on aquatic ecosystems. *J Photochem Photobiol B*, 46(98), pp.53–68.

- Hale, G.M. and Querry, M.R., 1973. Optical constants of water in the 200-nm to 200-um wavelength region. *Appl Optics*, 12(3), pp.555–563.
- Heaney, S.I. and Talling, J.F., 1980. Dynamic aspects of dinoflagellate distribution patterns in a small, productive lake. *J Ecol*, 68(1), pp.75–94.
- Jiang, Z., Vasil, A.I., Hale, J.D., Hancock, R.E.W., Vasil, M.L., and Hodges, R.S., 2008. Effects of net charge and the number of positively charged residues on the biological activity of amphipathic alpha-helical cationic antimicrobial peptides. *Biopolymers*, 90(3), pp.369–383.
- Kadir, K. and Nelson, K.L., 2014. Sunlight mediated inactivation mechanisms of *Enterococcus faecalis* and *Escherichia coli* in clear water versus waste stabilization pond water. *Water Res*, 50, pp.307–317.
- Kohn, T., Grandbois, M., McNeill, K., and Nelson, K.L., 2007. Association with natural organic matter enhances the sunlight-mediated inactivation of MS2 coliphage by singlet oxygen. *Environ Sci Technol*, 41(13), pp.4626–32.
- Latch, D.E. and McNeill, K., 2006. Microheterogeneity of singlet oxygen distribution in irradiated humic acid solutions. *Science*, 311, pp.1743–1747.
- Leech, D.M., Padeletti, A. and Williamson, C.E., 2005. Zooplankton behavioral responses to solar UV radiation vary within and among lakes. *J Plankton Res*, 27(5), pp.461–471.
- Malik, Z., Ladan, H. and Nitzan, Y., 1992. Photodynamic inactivation of Gram-negative bacteria and possible solutions. *J Photochem Photobiol B*, 14(3), pp.262–266.
- Malloy, K.D., Holman, M.A., Mitchell, D., and Detrich, H.W., 1997. Solar UVB-induced DNA damage and photoenzymatic DNA repair in antarctic zooplankton. *P Natl Acad Sci USA*, 94(4), pp.1258–63.
- Mamone, L., Di Venosa, G., Gándara, L., Sáenz, D., Vallecorsa, P., Schickinger, S., Rossetti, M.V., Battle, A., Buzzola, F., and Casas, A., 2014. Photodynamic inactivation of Gram-positive bacteria employing natural resources. *J Photochem Photobiol B*, 133, pp.80–89.
- Maraccini, P.A., Wang, D., McClary, J.S., and Boehm, A.B., 2015. Growth-dependent photoinactivation kinetics of *Enterococcus faecalis*. *J Appl Microbiol*, 118, pp.1226–1237.
- Maraccini, P.A., Mattioli, M.C., Sassoubre, L.M., Cao, Y., Griffith, J.F., Ervin, J.S., Van De Werfhorst, L.C., and Boehm, A. B., 2016. Solar inactivation of enterococci and *Escherichia coli* in natural waters: Effect of water absorbance and depth. *Environ Sci Technol*, DOI 10.1021/acs.est.6b00505.
- Maraccini, P.A., Wenk, J. and Boehm, A.B., 2016. Photoinactivation of eight health-relevant bacterial species: Determining the importance of the exogenous indirect mechanism. *Environ Sci Technol*, DOI 10.1021/acs.est.6b00074.
- Najm, I., 2006. An alternative interpretation of disinfection kinetics. *J Am Water Works Ass*, 98(10), p.93.
- Nguyen, M.T., Jasper, J.T., Boehm, A.B., and Nelson, K.L., 2015. Sunlight inactivation of fecal indicator bacteria in open-water unit process treatment wetlands: Modeling endogenous and exogenous inactivation rates. *Water Res*, 83, pp.282–290.
- Romero, O.C., Straub, A.P., Kohn, T., and Nguyen, T.H., 2011. Role of temperature and Suwannee River

natural organic matter on inactivation kinetics of rotavirus and bacteriophage MS2 by solar irradiation. *Environ Sci Technol*, 45, pp.10385–11393.

Romero-Maraccini, O.C., Sadik, N.J., Rosado-Lausell, S.L., Pugh, C.R., Niu, X.-Z., Croue, J.-P., and Nguyen, T.H., 2013. Sunlight induced inactivation of human Wa and porcine OSU rotaviruses in the presence of exogenous photosensitizers. *Environ Sci Technol*, 47, pp.11004–11012.

Rosado-Lausell, S.L., Wang, H., Gutierrez, L., Romero-Maraccini, O.C., Niu, X.-Z., Gin, K.Y.H., Croue, J.-P., and Nguyen, T. H., 2013. Roles of singlet oxygen and triplet excited state of dissolved organic matter formed by different organic matters in bacteriophage MS2 inactivation. *Water Res*, 47(14), pp.4869–4879.

Schuch, A.P. and Menck, C.F.M., 2010. The genotoxic effects of DNA lesions induced by artificial UV-radiation and sunlight. *J Photochem Photobiol B*, 99(3), pp.111–6.

Schwarzenbach, R.P., Gschwend, P.M. and Imboden, D.M., 2002. *Environmental Organic Chemistry* 2nd Editio., Hoboken, New Jersey, USA: John Wiley & Sons, Inc.

Sinton, L.W., Hall, C.H., Lynch, P.A., and Davies-Colley, R. J., 2002. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. *Appl Environ Microbiol*, 68(3), pp.1122–1131.

Sinton, L.W., Davies-Colley, R.J. and Bell, R.G., 1994. Inactivation of enterococci and fecal coliforms from sewage and meatworks effluents in seawater chambers. *Appl Environ Microbiol*, 60(6), pp.2040–8.

Sinton, L.W., Finlay, R.K. and Lynch, P.A., 1999. Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. *Appl Environ Microbiol*, 65(8), pp.3605–3613.

Taheri-Araghi, S. and Ha, B.Y., 2007. Physical basis for membrane-charge selectivity of cationic antimicrobial peptides. *Phys Rev Lett*, 98(16), pp.5–8.

Thurston-Enriquez, J.A., Haas, C.N., Jacangelo, J., Riley, K., and Gerba, C.P., 2003. Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. *Appl Environ Microbiol*, 69(1), pp.577–582.

van Boekel, M.A.J.S., 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *Int J Food Microbiol*, 74(1-2), pp.139–59.

Whitman, R.L., Nevers, M.B., Korinek, G.C., and Byappanahalli, M.N., 2004. Solar and temporal effects on *Escherichia coli* concentration at a Lake Michigan swimming beach. *Appl Environ Microbiol*, 70(7), p.4276.

Wilhelm, S.W., Weinbauer, M.G., Suttle, C.A., and Jeffrey, W.H., 1998. The role of sunlight in the removal and repair of viruses in the sea. *Limnol Oceanogr*, 43, pp.586–592.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Experimental setup details and fitting information for all experiments

Table S2. Experimental setup details and fitting information for fit chosen via criteria detailed in Methods section, and comparison of \hat{k} -values

Table S3. Depth-averaged light irradiance, observed \hat{k} , $\hat{k}_{endogenous}$, and $\hat{k}_{exogenous}$

Table S4. Remake of Table S2 with inclusion of UVA light in light screening calculations

Table S5. Remake of Table S3 with inclusion of UVA light in light screening calculations

Figure S1. Photoinactivation curves of bacteria in various photosensitizers when irradiated with UVB-reduced simulated sunlight

Figure S2. Absorbance spectra of photosensitizers in the experimental solutions

Figure S3. Remake of Figure 3 with inclusion of UVA light in light screening calculations

Bacteria	$\hat{k}_{\text{endogenous}}$ (m ² MJ ⁻¹)		$k_{\text{endogenous}}$ (min ⁻¹)		Percent reduction of
	Full Spectrum	UVB Reduced	Full Spectrum	UVB Reduced	$k_{\text{endogenous}}$
<i>B. thetaiotaomicron</i>	12678 ± 3742	5008 ± 2467	1.17 ± 0.34	0.067 ± 0.028	94%
<i>C. jejuni</i>	3873 ± 953	3835 ± 667	0.35 ± 0.08	0.052 ± 0.009	85%
<i>Ent. faecalis</i>	1944 ± 347	1123 ± 293	0.18 ± 0.03	0.016 ± 0.004	91%
<i>E. coli</i> K12	1480 ± 547	974 ± 230	0.12 ± 0.05	0.012 ± 0.003	90%
<i>E. coli</i> O157:H7	1470 ± 142	914 ± 77	0.14 ± 0.01	0.012 ± 0.001	92%
<i>S. enterica</i>	1559 ± 317	874 ± 382	0.14 ± 0.01	0.011 ± 0.005	92%
<i>Staph. aureus</i>	2127 ± 496	4059 ± 1400	0.16 ± 0.04	0.046 ± 0.014	72%
<i>Strep. bovis</i>	3952 ± 1492	1172 ± 104	0.36 ± 0.14	0.017 ± 0.002	95%

Table 1. Endogenous photoinactivation rate constants, $\hat{k}_{\text{endogenous}}$ and $k_{\text{endogenous}}$, for bacteria irradiated by full spectrum simulated sunlight (taken from Maraccini *et al.* (2016b)) and by UVB-reduced simulated sunlight reported herein. Values shown represent the average of at least 3 experiments ± one standard deviation. The percent reduction from $k_{\text{endogenous}}$ under full spectrum simulated sunlight to UVB-reduced simulated sunlight is listed for all bacteria tested.

Figure Captions:

Figure 1. Average daily irradiance of UVB light, UVA light, and visible light at depths from the surface to 15 cm depth in wetland water (total organic carbon = 12.3 mg l^{-1} as C, San Joaquin Creek, Irvine, CA, $33.6694^{\circ} \text{ N}$, $117.8231^{\circ} \text{ W}$) on September 14th, 2015. The light intensity at different depths was calculated from the water absorbance, light intensity at the water surface, and the solar zenith angle, as described in a previous study (Maraccini *et al.* 2016a). The water absorbance was measured with an Uvikon XL Spectrophotometer (BioTek Instruments, Winooski, VT) and can be mainly attributed to the absorption of the dissolved natural organic matter present in the wetland water while the absorbance of the water itself is small (between $0.025\% \text{ cm}^{-1}$ at 550 nm and approximately $1\% \text{ cm}^{-1}$ at 280 nm) (Hale and Querry 1973). The light intensity incident to the water surface and the solar zenith angle were obtained from the Simple Model of the Atmospheric Radiative Transfer of Sunshine (SMARTS).

Figure 2. Light spectra of the natural sunlight (solid line, midday summer in Palo Alto, CA) and solar simulator with the UVB cutoff filter (dashed line).

Figure 3. Inactivation rate constants of Gram-positive facultative anaerobes, Gram-negative facultative anaerobes, an obligate anaerobe, and a microaerophile in 1 mmol l^{-1} carbonate buffer saline with various photosensitizers (CBS = no photosensitizer, MB = $0.5 \text{ } \mu\text{mol l}^{-1}$ methylene blue, RB = $0.5 \text{ } \mu\text{mol l}^{-1}$ rose bengal, Nitrite = $200 \text{ mg l}^{-1} \text{ NO}_2^-$, SRNOM = 20 mg l^{-1} as C Suwannee River natural organic matter, iDOM = 20 mg l^{-1} as C dissolved organic matter isolated from a treatment wetland) when irradiated with simulated sunlight passing through a UVB cutoff filter. Values are averages and standard errors from pooled biological replicates; note that pooled values were not used in hypothesis testing and are shown here for visualization. The stripped

bars are the control solutions (CBS), the grey bars indicate the photosensitizer increased \hat{k} , and the white bars indicate there was no change in \hat{k} compared to the control. The \hat{k} values, standard deviations, and 95% confidence intervals for individual experiments may be found in the Supporting Information (Tables S1 and S2).





